

CLAIMS

We claim:

1. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising:

5 a) providing a first probe comprising:

i) an upstream universal priming site (UUP);

10 ii) an adapter sequence;

15 iii) a first target-specific sequence comprising a first base at a readout position; and

20 iv) a downstream universal priming site (DUP);

b) contacting said first probe with said target sequence under conditions whereby only if said first base is perfectly complementary to a nucleotide at said detection position is a first hybridization complex formed;

c) removing non-hybridized first probes;

25 d) denaturing said hybridization complex;

e) amplifying said first probe to generate a plurality of amplicons;

f) contacting said amplicons with an array of capture probes; and

30 g) determining the nucleotide at said detection position.

2. A method according to claim 1 wherein said amplicons comprise a label.

3. A method according to claim 1 further comprising:

35 a) providing a second probe comprising:

i) an upstream universal priming site (UUP);

40 ii) an adapter sequence;

45 iii) a second target-specific sequence comprising a second base at said readout position; and

50 iv) a downstream universal priming site (DUP);

b) contacting said second probe with said target sequence under conditions whereby only if said second base is perfectly complementary to a nucleotide at said detection

55 position is a second hybridization complex formed;

c) removing non-hybridized second probes;

60 d) denaturing said second hybridization complex;

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e) amplifying said second probe to generate a plurality of amplicons;  
f) contacting said amplicons with an array of capture probes; and  
g) determining the nucleotide at said detection position.

4. A method of determining the identification of a nucleotide at a detection position in a target  
5 sequence comprising:

a) providing a plurality of readout probes each comprising:  
i) an upstream universal priming site (UUP);  
ii) an adapter sequence;  
iii) a target-specific sequence comprising a unique base at a readout  
10 position; and  
iv) a downstream universal priming site (DUP);  
b) contacting said detection probes with said target sequence under conditions  
whereby only if said base at said readout position is perfectly complementary to a  
15 nucleotide at said detection position is a first hybridization complex formed;  
c) removing non-hybridized first probes;  
d) denaturing said first hybridization complex;  
e) amplifying said detection probes to generate a plurality of amplicons;  
f) contacting said amplicons with an array of capture probes; and  
g) determining the nucleotide at said detection position.

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5. A method of determining the identification of a nucleotide at a detection position in a target  
sequence comprising a first target domain comprising said detection position and a second target  
domain adjacent to said detection position, wherein said method comprises:  
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a) hybridizing a first ligation probe to said first target domain, said first ligation probe  
25 comprising:  
i) an upstream universal priming site (UUP); and  
ii) a first target-specific sequence; and  
b) hybridizing a second ligation probe to said second target domain, said second  
ligation probe comprising:  
30 i) a downstream universal priming site (DUP); and  
ii) a second target-specific sequence comprising a first base at an  
interrogation position;  
wherein if said first base is perfectly complementary to said nucleotide at said

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5 detection position a ligation complex is formed and wherein at least one of said first and second ligation probes comprises an adapter sequence;

- c) removing non-hybridized first probes;
- d) providing a ligase that ligates said first and second ligation probes to form a ligated probe;
- e) amplifying said ligated probe to generate a plurality of amplicons;
- f) contacting said amplicons with an array of capture probes; and
- g) determining the nucleotide at said detection position.

6. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

- a) hybridizing a first ligation probe to said first target domain, said first ligation probe comprising:

- i) an upstream universal priming site (UUP); and
  - ii) a first target-specific sequence; and

- b) hybridizing a second ligation probe to said second target domain, said second ligation probe comprising:

- i) a downstream universal priming site (DUP); and
  - ii) a second target-specific sequence comprising a first base at an interrogation position;

20 wherein if said first base is perfectly complementary to said nucleotide at said detection position a ligation complex is formed and wherein at least one of said first and second ligation probes comprises an adapter sequence;

- c) removing non-hybridized first probes;

- d) providing a ligase that ligates said first and second ligation probes to form a ligated probe;

25 e) hybridizing said ligated probe to a rolling circle (RC) sequence comprising:

- i) an upstream priming sequence; and
  - ii) a downstream priming sequence;

- f) providing a ligase that ligates said upstream and downstream priming sites to form a circular ligated probe;

30 g) amplifying said circular ligated probe to generate a plurality of amplicons;

- f) contacting said amplicons with an array of capture probes; and

- g) determining the nucleotide at said detection position.

7. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

5 a) hybridizing a rolling circle (RC) probe to said target sequence, said RC probe comprising:

- i) an upstream universal priming site (UUP); and
- ii) a first target-specific sequence;
- iii) a second target-specific sequence comprising a first base at an interrogation position; and
- iv) an adapter sequence;

10 wherein if said first base is perfectly complementary to said nucleotide at said detection position a ligation complex is formed;

- c) providing a ligase that ligates said first and second ligation probes to form a ligated probe;
- d) amplifying said ligated probe to generate a plurality of amplicons;
- e) contacting said amplicons with an array of capture probes; and
- f) determining the nucleotide at said detection position.

15 8. A method according to claim 7, further comprising removing non-hybridized RC probe.

20 9. A method according to claim 1, 4, 5, 6 or 8 wherein said removing comprises:

- a) enzymatically adding a binding ligand to said target sequence;
- b) binding a hybridization complex comprising said target sequence comprising said binding ligand to a binding partner immobilized on a solid support;
- c) washing away unhybridized probes; and
- d) eluting said probe off said solid support.

25 10. A method according to claim 1, 4, 5, 6 or 8 wherein said removing is done using a double-stranded specific moiety.

11. A method according to claim 10 wherein said double-stranded specific moiety is an intercalator attached to a support.

*Sub Bb* 12. A method according to claim 9 wherein said support is a bead.

13. A method according to claim 1, 4, 5, 6 or 7 wherein said amplifying is done by:

- a) hybridizing a first universal primer to said UUP;
- b) providing a polymerase and dNTPs such that said first universal primer is extended;
- c) hybridizing a second universal primer to said DUP;
- d) providing a polymerase and dNTPs such that said second universal primer is extended; and
- e) repeating steps a) through d).

10 14. A method according to claim 1, 4, 5, 6 or 7 wherein said array comprises:

- a) a substrate with a patterned surface comprising discrete sites; and
- b) a population of microspheres comprising at least a first subpopulation comprising a first capture probe and a second subpopulation comprising a second capture probe.

15 15. A method according to claim 14 wherein said discrete sites comprise wells.

16. A method according to claim 14 or 15 wherein said substrate comprises a fiber optic bundle.

17. A method of determining the identification of a nucleotide at a detection position in a genomic target sequence comprising:

- a) attaching a library of genomic target sequences to a solid support;
- b) adding at least one probe and an enzyme to form an extended primer;
- c) denaturing said extended primer from said target sequence;
- d) hybridizing said extended primer to an array comprising capture probes; and
- e) determining said nucleotide at said detection position.

20 18. A method according to claim 17 further comprising removing unhybridized probes.

25 19. A method according to claim 1, 4, 5, 6 or 7, further comprising providing a support on which the target sequence is immobilized.

*Sub Bf* 20. A method according to claim 19, wherein said non-hybridized first probes are removed without

removing said target sequence from said support.

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A method according to claim 1, 4, 5, 6 or 7, further comprising attaching said target sequence to a support.

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A method according to claim 21, wherein said target sequence is attached to said support by a method selected from the group consisting of labeling said target sequence with a functional attachment moiety, absorption of said target sequence on a charged support, direct chemical attachment of said target sequence to said support and photocrosslinking said target sequence to said support.

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A method according to claim 1, 4, 5, 6 or 7, wherein said support is selected from the group consisting of paper, plastic and tubes.

24. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising:

- a) providing a support on which the target sequence is immobilized;
- b) providing a first probe comprising:
  - i) an upstream universal priming site (UUP);
  - ii) an adapter sequence;
  - iii) a first target-specific sequence comprising a first base at a readout position; and
  - iv) a downstream universal priming site (DUP);

20 c) contacting said first probe with said target sequence under conditions whereby only if said first base is perfectly complementary to a nucleotide at said detection position is a first hybridization complex formed;

d) removing non-hybridized first probes;

e) denaturing said hybridization complex;

f) amplifying said first probe to generate a plurality of amplicons;

g) contacting said amplicons with an array of capture probes; and

h) determining the nucleotide at said detection position

25 ✓ 25. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising:

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- a) providing a support on which the target sequence is immobilized;
- b) providing a plurality of readout probes each comprising:
  - i) an upstream universal priming site (UUP);
  - ii) an adapter sequence;
  - iii) a target-specific sequence comprising a unique base at a readout position; and
  - iv) a downstream universal priming site (DUP);
- c) contacting said detection probes with said target sequence under conditions whereby only if said base at said readout position is perfectly complementary to a nucleotide at said detection position is a first hybridization complex formed;
- 10 d) removing non-hybridized first probes;
- e) denaturing said first hybridization complex;
- f) amplifying said detection probes to generate a plurality of amplicons;
- 15 g) contacting said amplicons with an array of capture probes; and
- h) determining the nucleotide at said detection position.

✓ 26. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

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- a) providing a support on which the target sequence is immobilized;
- b) hybridizing a first ligation probe to said first target domain, said first ligation probe comprising:
  - i) an upstream universal priming site (UUP); and
  - ii) a first target-specific sequence; and
- c) hybridizing a second ligation probe to said second target domain, said second ligation probe comprising:
  - i) a downstream universal priming site (DUP); and
  - ii) a second target-specific sequence comprising a first base at an interrogation position;

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wherein if said first base is perfectly complementary to said nucleotide at said detection position a ligation complex is formed and wherein at least one of said first and second ligation probes comprises an adapter sequence;

- d) removing non-hybridized first probes;
- e) providing a ligase that ligates said first and second ligation probes to form a ligated probe;

- f) amplifying said ligated probe to generate a plurality of amplicons;
- g) contacting said amplicons with an array of capture probes; and
- h) determining the nucleotide at said detection position.

✓ 27. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

- a) providing a support on which the target sequence is immobilized;
- b) hybridizing a first ligation probe to said first target domain, said first ligation probe comprising:

- i) an upstream universal priming site (UUP); and
- ii) a first target-specific sequence; and

- c) hybridizing a second ligation probe to said second target domain, said second ligation probe comprising:

- i) a downstream universal priming site (DUP); and
- ii) a second target-specific sequence comprising a first base at an interrogation position;

wherein if said first base is perfectly complementary to said nucleotide at said detection position a ligation complex is formed and wherein at least one of said first and second ligation probes comprises an adapter sequence;

- d) removing non-hybridized first probes;
- e) providing a ligase that ligates said first and second ligation probes to form a ligated probe;
- f) hybridizing said ligated probe to a rolling circle (RC) sequence comprising:

- i) an upstream priming sequence; and
- ii) a downstream priming sequence;

- g) providing a ligase that ligates said upstream and downstream priming sites to form a circular ligated probe;
- h) amplifying said circular ligated probe to generate a plurality of amplicons;
- i) contacting said amplicons with an array of capture probes; and
- j) determining the nucleotide at said detection position.

✓ 28. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

- a) providing a support on which the target sequence is immobilized;
- b) hybridizing a rolling circle (RC) probe to said target sequence, said RC probe comprising:
  - i) an upstream universal priming site (UUP); and
  - ii) a first target-specific sequence;
  - iii) a second target-specific sequence comprising a first base at an interrogation position; and
  - iv) an adapter sequence;
- wherein if said first base is perfectly complementary to said nucleotide at said detection position a ligation complex is formed;
- c) providing a ligase that ligates said first and second ligation probes to form a ligated probe;
- d) amplifying said ligated probe to generate a plurality of amplicons;
- e) contacting said amplicons with an array of capture probes; and
- f) determining the nucleotide at said detection position.

29. A method according to claim 28, further comprising removing unhybridized RC probe.

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